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Histological measures of digestibility of fodder plants linked to their genetic variation

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Key words: Morphological composition, digestibility, Medicago, selection, genetic variability

1 SUMMARY

By measuring part of ligno-cellulosic tissues in the histological section of stems and of leaves with an optical microscope ruler. The biometric parameters reported were : leaves on stems or lignified tissues on non-lignified tissues, determined the population most digested by ruminants. This is, without passing by *in vivo* or *in vitro* digestibility. Photos of histological sections of stem show that genetic variations of digestibility are linked to xylem sclerenchyma proportion (lignified tissues) and to non-lignified tissues proportion (medullary parenchyma and cortical parenchyma).This proved that digestibility of stems is lower than leaves, top of the stems is more digestible than the bottom ones and perennial species are less digestible than annual ones. In addition, there is a close relationship between plant morphology (report leaves-stems)and the rate of lignified tissues and non-lignified. Comparison of non-lignified tissues to lignified ones, can be multiplied by number of bundles and shows that result is higher in *M.muricoleptis* than in *M .ciliaris,* respectfully 187.5 and 77. Therefore the method it is based on simple measurements and calculations by the optical microscope.

2 INTRODUCTION

When a fodder plant is ageing, leaves proportion declines to stems' benefit. The progressive lignifications of those ones affects energy value, nitrogen value, mineral value and digestibility. This decrease is even more rapid as growth is rapid itself (Dominique et al, 2014).According Jarrige (1981) and Peyronie (1982), the report leaves / stems can be a good indicator of quality in alfalfa. Knowing that cells from proliferation of meristematic cells progressively differentiates in adult tissues. The cell walls modify their chemical character and thicken at the expense of cytoplasm, which in certain cells disappears even completely, case of xylem (Rober et Marie. 2000). Then, as a result of microscopic scale that content in cytoplasm components decreases and that content in parietal components increases, notably in lignin (Jarrige.,1989) However, if these fodders arrived in full into the rumen, it would be settled only more slowly by microbes because of barriers constituted by thick cuticle which covers certain epidermis, and lignified tissues. The chemical composition of a plant product its morphological composition. This varies according to the organ, the botanical family and species of the plant and the stage (Porqueddu 2000). However, if these fodders arrived in full into the rumen, it would be settled only more slowly by microbes because of barriers constituted by thick cuticle which covers certain epidermis, and lignified tissues. The digestive chewing begins to breaking up these barriers. Release soluble components of naked internal tissues, which they will be exposed to microbe's invasion of rumen (Jarrige et al, 1995, Hugues, 2008). Therefore, two simultaneous phenomena are carried out during growth; a decrease of leaves proportion in fodder in favour of stems,

which are less digestible, and a decrease of stems digestibility. There is so, a negative relationship between biomass produced and digestibility of the whole plant. For a long time, measurement of digestibility in laboratory allows treating a large number of samples. The gravimetric method using enzymes (enzymatic solubility method) which consists to treat samples by pepsin then by cellulases. It is highly repeatable and correlated with in vitro digestibility using rumen juice and in vivo digestibility measured by animals. All these methods are long and expensive; limit their use from a certain samples number (Aufrère et *al* .,2007). For this reason, since the digestibility in ruminant is stopped by the excessive presence of cellulose and lignin in fodder, we focused on tissues that include these two chemical elements (Xylem and phloem) to appreciate digestibility at early flowering stage. Xylem and phloem are two tissues whose cells are lignified to support their mechanical functions, making easy water movement (William et Hopkins. 2003). Furthermore, dosing of fibre content of these tissues in the whole plant (NDF for Neutral Detergent Fibre, ADF for Acid Detergent Fibre, ADL for Acid Detergent Lignin) are widely used by scientific community. Results with this method in *Medicago* offer 43% of vegetable walls dry matter whose 20% are constituted by raw lignin, 32% by hemicelluloses and 48% by cellulose (Mauriès, 1994). In this article we have performed histological sections at level of Medicago' stems. By calculations and measures of ligno-cellulosic tissues by using optical microscope the plant's digestibility is evaluated

3 MATERIALS AND METHODS

On each genotype, 3 dominant stems have been sampled in early stage bloom and at initial pod set, to perform histological analyses and quantification by measurement and calculation. On these 3 stems, a portion of 2 cm length has been sampled every 5 cm going from apex to the stems bottom, except the top of the stems where the first cm has been cut. Stems height and their number by linear meter have been noted. As for study of genetic diversity between species of Medicago, histological analysis has been performed mainly at the bottom of stems. Then it is possible to calculate proportion of different tissues according to stems 'radius in order to express distribution of different tissues in the same unit. Treatment with X 4o magnification allows obtaining radius of the transversal section. Different stems tissues proportions are calculated by the following manner:

Bark proportion = bark thickness / section radius
Proportion of xylem = width of xylem / section radius.
Proportion of medullar parenchyma = thickness of medullar parenchyma / section radius.

Therefore, we have developed histological sections at different level of stems, calculate report tissues on non-lignified tissue and lignified tissues Diameter of the stems was measured before to make sections in early flowering stage. This stage is appreciated at two (2) buds with open flowers in order to eliminate all differences caused by maturity.

3.1 Vegetable material

**M.ciliaris*
**M .muricoleptis*
**M.intertexta*
**M.tuncatula*
**M.sativa*

3.2 The manual colouring method of cuts : Sections were put in bleach water (20 mn) to empty the cells.Sections were then washed in acetic acid (1 mn)and coloured with green Methyl (5 mn)and red Congo (10 mn).

3.3 Measure method: An optical microscope was used whose binocular magnifier was maintained with a rule allowing measurement of tissues 'thickness. After by combination of both rules and both units, the unit corresponded to 7.5µm (Schéma1)

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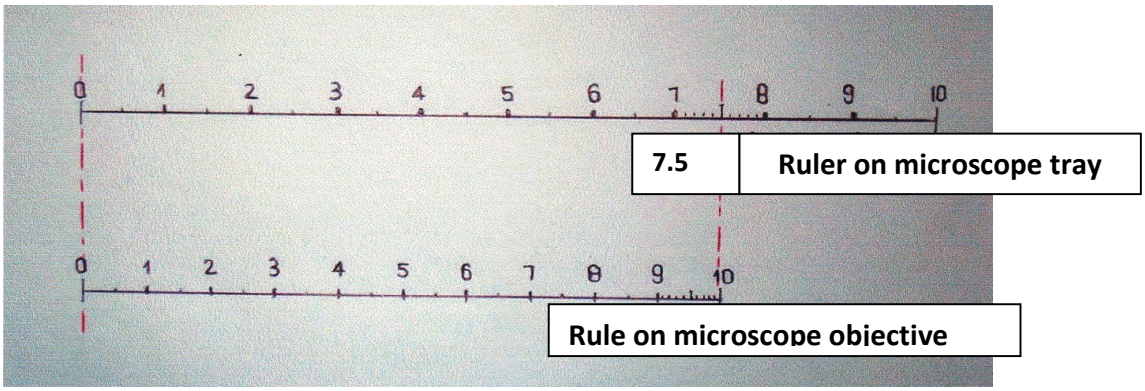

Schéma.1- After by combination of both rules and both units, unit Corresponds to 7.5 µm.

4 RESULTS

In all leaves species studied and at two different stages: early flowering and initial pods set (photos 1.2.), a histological cut shows only one woody bundle Hence, leaves' digestibility do not evaluate with age of the plant. This agrees with bibliographic data showing that digestibility of leaves is high (Selmi et *al*, 2010).

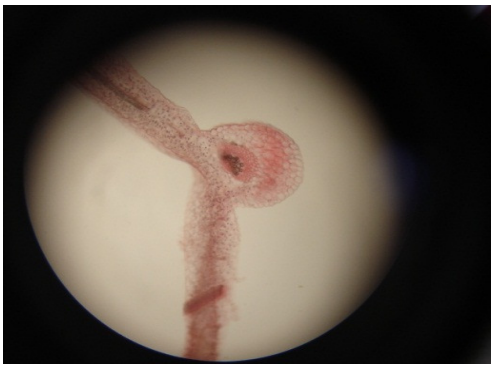

Photo 1: Transversal cut of leaf of *M.muricoleptis*

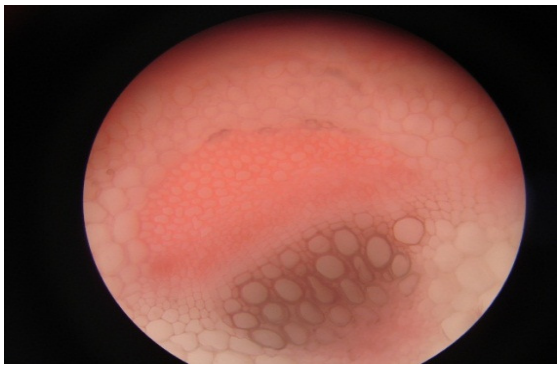

Photo 2: Detail of leaf bundle of *M muricoleptis* observed before early flowering stage

According to Table1, The report of non-lignified tissues on lignified tissues multiplied by number of bundles shows that is high in *M muricoleptis* (77).which is consistent with observations made on photos (3.4). The sclerenchyma capsule is clear in photo 5. The secondary tissues are well differentiated in early flowering stage. This is more evident on the circle that forms xylem in photos 5.

Table1: Results of parameters measures of digestibility on the stem bottom in *M.muricoleptis.*

Species	Number of bundles	Thickness of bundles (µm)	Thickness of lignified tissues (µm)	Thickness of non lignified tissues	Report Tissues non lignified Tissues	Report Leaves Stems

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					lignified	
M .muricoleptis	11	165	75	525	7	0,95

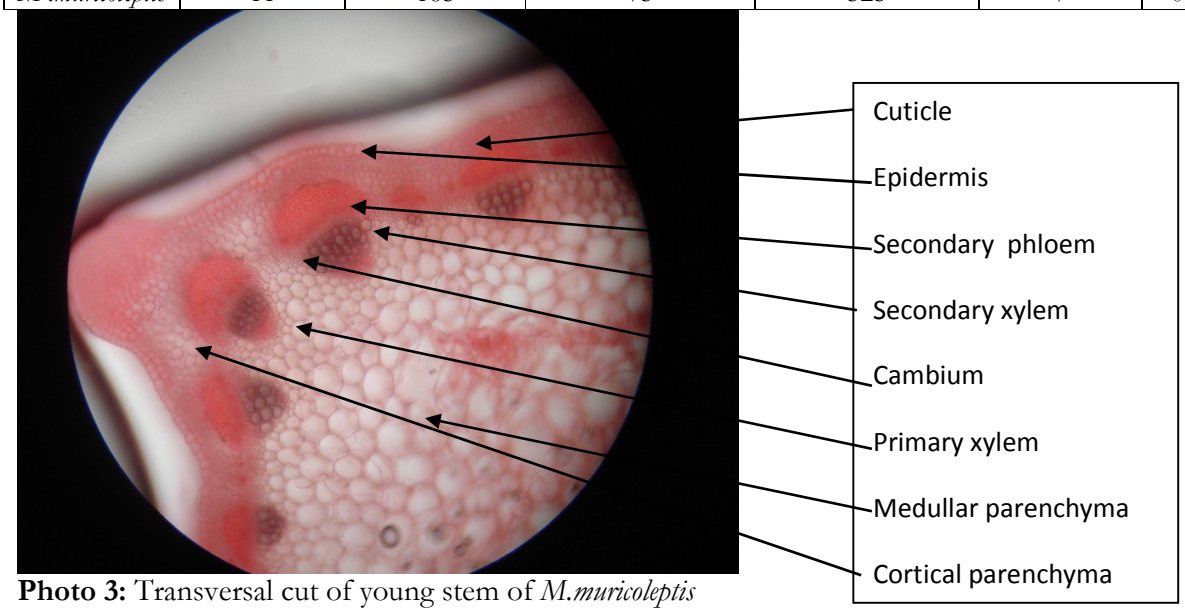

Photo 3: Transversal cut of young stem of *M.muricoleptis*

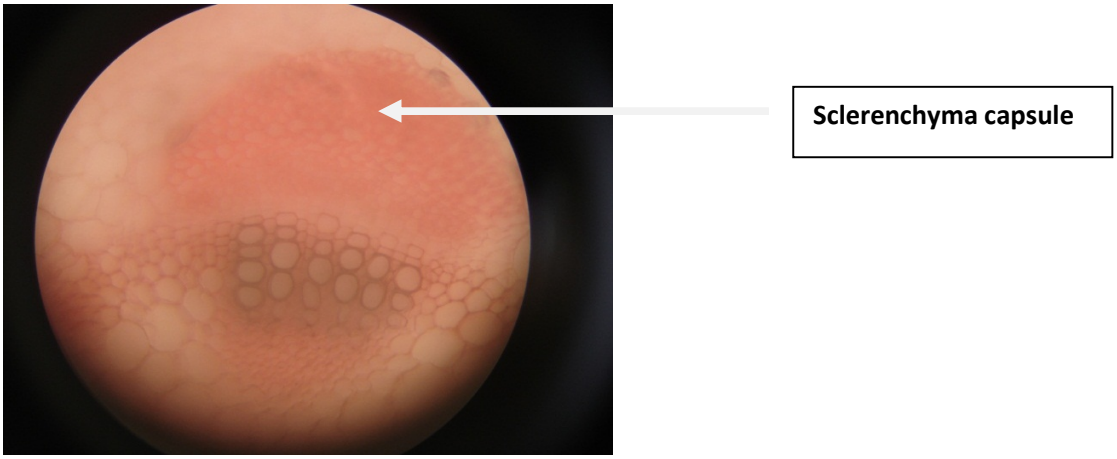

Photo 4: Details of woody bundle at level of young stem *M. Muricoleptis*

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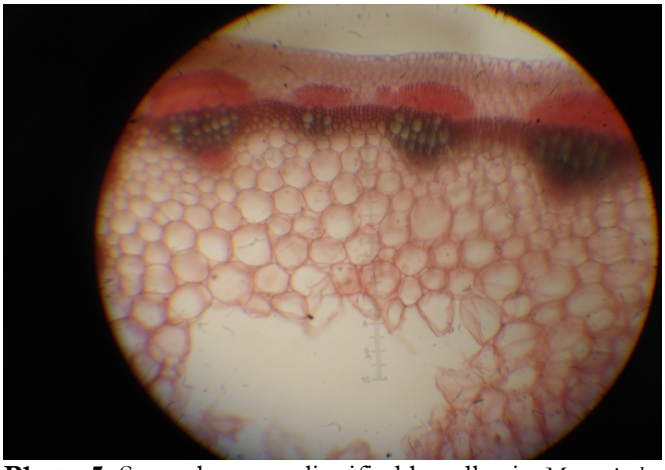

Photo 5: Space between lignified bundles in *M.muricoleptis* at early flowering stage

According to Table 2 and microscopic observations in *M.ciliaris* species, the same report calculated in *M.muricoleptis* gives value of 187.5 above that found in last one.. A fibrous tissue that we have not found in other species is developed, it is tangential Collenchymas. It is well differentiated at early flowering stage (photo 8). After pods set, another tissue is clearly developed at level of photo 9, which is sclerenchyma. These both tissues have increased part of fibres in the species; this has decreased its digestibility.

Table 2: Results of parameters measures of the digestibility at the stem base in *M.ciliaris*

Species	Number of bundles	Thickness of bundles (µm)	Thickness of tissues lignified (µm)	Thickness of tissues non lignified	Report Tissues non lignified / Lignified tissues	Report Leaves / Stems
M .ciliaris	15	150	45	562,5	12,5	0,88

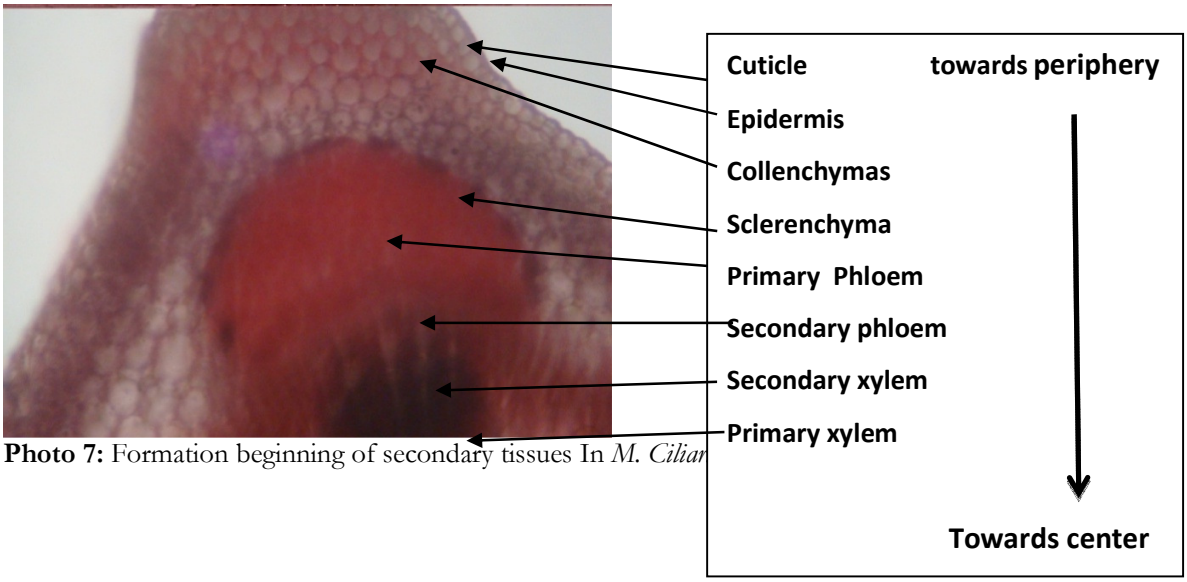

Photo 7: Formation beginning of secondary tissues In *M. Ciliar*

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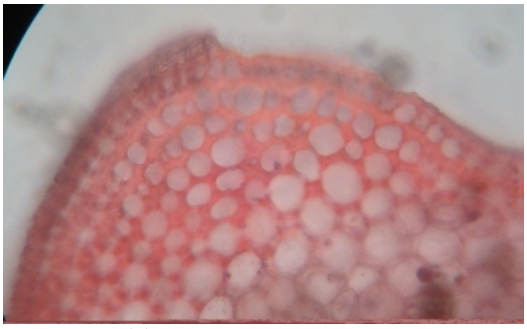

Photos 8: Tangential Collenchymas

According data of table 3 number of bundles is higher at the base than at the top of species stem . *M .truncatula* .which will increase part of lignin at this level Consequently, extreme thickness of the stem is larger, and so thickness of bundles (Photo 10 et 11). At this spot, we have secondary tissues, which are forming within bundle (Photo 9). By linking morphological parameter number of stem and histological parameter tissues report non lignified tissues on lignified tissues, we obtain the following result: At the top: stem number x report = 4.8, The base : number of stem x report = 7.7. The result shows that the base of the stem is the double of the top.

Table 3: Results of measurements parameters of digestibility at the top and at the stem base in *M .truncatula.*

The Stem	Number of bundles	Thickness of xylem (µm)	Thickness between bundles (µm)	Tissues non lignified (µm)	Lignified tissues (length of bundles (µm)	Tissues lignified rapport on non lignified
The top	12	112. 5	30	450	180	0,4
The base	14	180	15	405	225	0,55

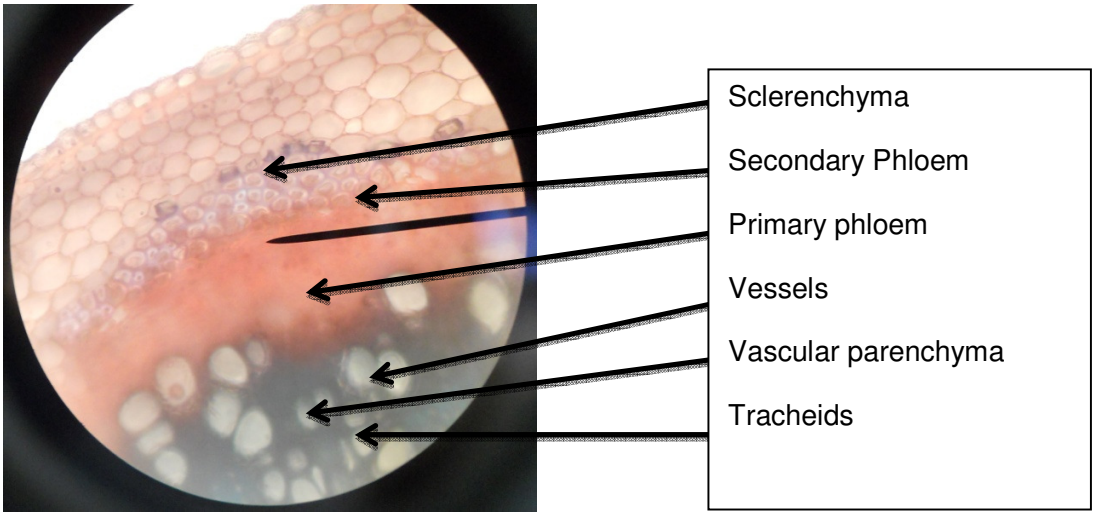

Photo 9:. Early fibres formation of sclerenchyma in *M.ciliaris* after pods setting.

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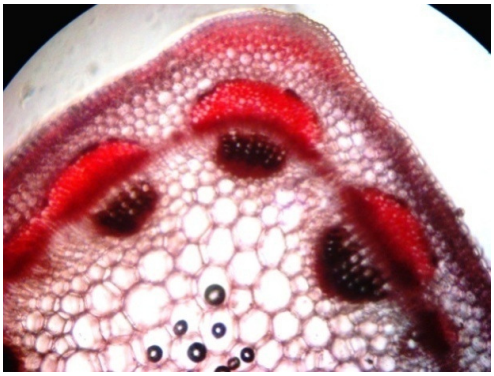
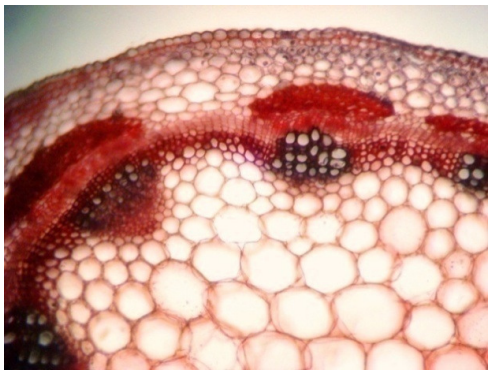

Photos 10 and 11:. Cut on top and on base of stem *M.truncatula*

Table 4 shows that stem base in early flowering stage in *M.intertexta is* more lignified than its apex. The woody bundles number is higher; thickness of woody tissues is larger (250 µm against 130 µm). Microscopic observations confirm these results by presence of secondary tissues (capsule of secondary sclerenchyma, xylem). Formula which links morphological parameter (stem number) to histological parameter (report leaf on stem) records a difference between apex and stem base with about only one point in *M.intertexta's* species (Apex : number of stem x report leaf on stem = 6.89. Base : number of stem x leaf report on stem = 7.84).

Table 4: Results of parameters measures of digestibility at apex and at the base of stem *M.intertexta.*

Stem	Number of bundles	Thickness of xylem (µm)	Thickness between bundles (µm)	Tissues non lignified (µm)	Lignified tissues (length of bundles (µm)	Tissues lignified rapport on non lignified
Apex	13	97,5	67.5	337.5	130	0,53
The base	14	150	90	450	250	0,56

Comparison of annual species to perennial species during its first cycle shows us that secondary tissues are more developed. Photo (12) shows a clear secondary setting at level of stem's base of *M.sativa* compared to annual species of the same kind. That is confirmed by report measure of lignified tissues on non-lignified, which is by far the highest to those of annual species (Table 5). Stem number x leaf report on stem gives result of 12.88.

Table 5: Results of parameters measures of digestibility at stem's base in *M. sativa.*

Stem	Number of bundles	Thickness of xylem (µm)	Thickness between bundles (µm)	Tissues non lignified (µm)	Lignified tissues (length of bundles (µm)	Tissues lignified rapport on non lignified
Base	14	105	97.5	202.5	187.5	0,92

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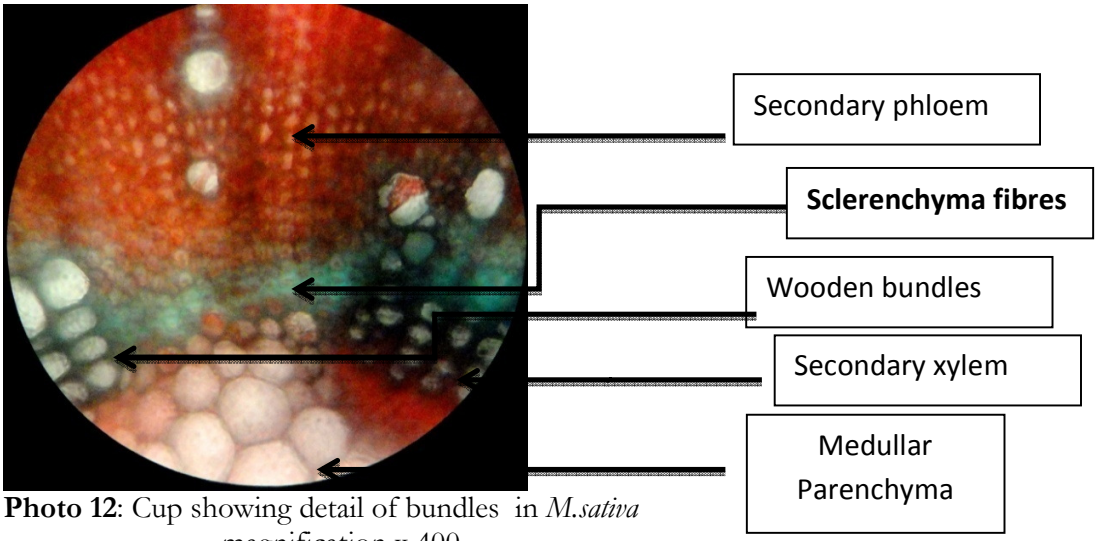

Photo 12: Cup showing detail of bundles in *M.sativa* magnification x 400

CONCLUSION

This appreciation method of digestibility is easy to perform. It is based on the assessment and measurements of lignified tissues under an optical microscope at a given good physiological stage of the plant and at the stem and not the leaf. .This last is completely digested by ruminants (Jarrige., 1981). This method allowed us to have in Medicago many results. By the high presence of lignified tissues at the stems digestibility of the whole plant depends on the digestibility of the stems; digestibility of the stems is less than that of the leaves;. The apex of stem is more digestible than stem's base. From the same rod there is close relationship between plant's morphology (report leaf stems, stem number) and rate of lignified tissues and non lignified of chemical composition of the plant (content in cellulose but above ll in lignin content). Between the same organ (stem), or same stage (early flowering) in both species there is significant differences of lignifications (*M muricoleptis* more lignified than *M .ciliaris)* however, capsule of sclerenchyma is more developed in *M muricoleptis* than in *M ciliaris* .We can say digestibility is higher with a report leaves on high stems as the leaves have very few woody tissues .A report leaves on high stalks corresponds to a mass of woody tissues report on high lignified tissues. The perennial species has more secondary tissue that annual species so it is less digestible. The two reports mentioned above (leaves on stems and woody tissues of non-woody tissues) are high with early physiological stage.

PERSPECTIVES

Select plants having a good rate of digestibility.

BIBLIOGRAPHY:

H Selmi, A Gasmi-Boubaker, W Mehdi, B Rekik, Y Ben Salah et H Rouissi., 2010. Composition chimique et digestibilité *in vitro* des feuilles d'*Hedysarum coronarium* L, *Medicago truncatula* L ,*Pisum sativum* L et *Vicia sativa* L. www.lrrd.org/lrrd22/6/selm22116.htm

Henri-Dominique Klein, Georges Rippstein, Johann Huguenin ., 2014. Les cultures fourragères .Collections agricultures tropicale 256p.

Hugues Tezenas du Montcel., 2008. Alimentation des ruminants. 116p.

J. Aufrère, R. Baumont, L. Delaby, J.-R. Peccatte, J. Andrieu, J.-P. Andrieu, J.-P. Dulphy., Prévision de la digestibilité des fourrages par la méthode pepsine-cellulase. Le point sur les équations proposées. INRA Prod. Anim.,2007, 20 (2), 129-136

Jarrige J., 1981. Précision de la valeur alimentaire des ruminants INRA. France. 13-40.

Jarrige.R, Y.Ruckebusch,C. Demarquilly,M-H .Farce,M.Journet.,1995 :Nutrition des ruminants domestiques .Ingestion et digestion. INRA, Paris.921p.

Lapeyronie A., 1982. Les productions fourragères méditerranéennes. G.P.M. et Larose. 245

Mathieu Mauriès., 1994 : La luzerne aujourd'hui. ed. France agricole 254p

Par William G. Hopkins., 2003. Physiologie végétale. https://books.google.dz/books?isbn=2744500895

Porqueddu C., 2000. Screening germplasm and varieties for quality: constraints and potentials in annual medics. Cahier Opti. Medi., serie A., 45 : 89-98.